

What is claimed is:

1. An isolated silk polypeptide comprising a plurality of repetitive units and a non-repetitive hydrophilic amino acid domain.
2. The silk polypeptide of claim 1, wherein at least two repetitive units are present in a head-to-tail configuration.
3. The silk polypeptide of claim 1, wherein the repetitive units are present in a head-to-tail configuration.
4. The silk polypeptide of claim 1, wherein at least two repetitive units are present in a head-to-head configuration.
5. The silk polypeptide of claim 1, wherein all the repetitive units are present in a head-to-head configuration.
6. The silk polypeptide of claim 1 comprising at least about 2 to about 4 repetitive units.
7. The silk polypeptide of claim 1 comprising at least about 5 to about 10 repetitive units.
8. The silk polypeptide of claim 1 comprising at least about 10 to about 50 repetitive units.
9. The silk polypeptide of claim 1 comprising at least about 50 to about 100 repetitive units.
10. The silk polypeptide of claim 1, wherein at least two of the repetitive units are contiguous.
11. The silk polypeptide of claim 10, wherein the repetitive units are contiguous.

12. The silk polypeptide of claim 1, wherein at least two of the repetitive units are separated by an amino acid spacer.

5 13. The silk polypeptide of claim 12, wherein the repetitive units are separated from each other by an amino acid spacer.

14. The silk polypeptide of claim 12, wherein the amino acid spacer is 1 to about 10 amino acids in length.

10 15. The silk polypeptide of claim 1, wherein the repetitive units comprise amino acid sequences forming a secondary structure selected from the group consisting of: β -turn spiral, crystalline β sheet, and 3_{10} helix.

15 16. The silk polypeptide of claim 1, wherein a repetitive unit comprises a repetitive unit found within an spider or insect silk polypeptide.

17. The silk polypeptide of claim 1, wherein each repetitive unit independently comprises a repetitive unit found within *Nephila clavipes* or *Araneus diadematus* spider silk polypeptides or *Bombyx mori* cocoon silk polypeptides.

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18. The silk polypeptide of claim 1, wherein the repetitive units comprise iterated peptide motifs selected from the group consisting of the amino acid sequences identified as SEQ ID NOS:4-27.

25 19. The silk polypeptide of claim 1, wherein the amino acid sequence of each repetitive unit is independently selected from the amino acid sequences of repetitive units found within the group consisting of ADF-1, ADF-2, ADF-3, ADF-4, ABF-1, MaSpI, MaSpII, MiSpI, MiSpII, and Flag.

30 20. The silk polypeptide of claim 19, wherein the amino acid sequence of each repetitive unit is selected from the group of amino acid sequences identified as SEQ ID No:1, SEQ ID No:2, and SEQ ID No:3.

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21. The silk polypeptide of claim 19, wherein at least one of the native repetitive regions has an amino acid sequence that is in a reversed order in comparison to the naturally-occurring amino terminus to carboxyl terminus amino acid sequence.

5 22. The silk polypeptide of claim 1, wherein the repetitive units comprise a plurality of iterated peptide motifs selected from the group consisting of: GPG(X)_n , $(\text{GA})_n$, A_n , and GGX ,

where X represents the amino acid A, Q, G, L, S, Y or V, and

n represents an integer from 1 to about 8.

10 23. The silk polypeptide of claim 1, wherein at least two of the repetitive units have identical amino acid sequences.

15 24. The silk polypeptide of claim 1, wherein the repetitive units have non-identical amino acid sequences.

25. The silk polypeptide of claim 1, wherein the non-repetitive hydrophilic amino acid domain is towards the carboxyl terminus with respect to the repetitive units.

20 26. The silk polypeptide of claim 1, wherein the non-repetitive hydrophilic amino acid domain is towards the amino terminus with respect to the repetitive units.

27. The silk polypeptide of claim 1, wherein the non-repetitive hydrophilic amino acid domain is between two of the repetitive units.

25 28. The silk polypeptide of claim 27, further comprising a proteolytic site, wherein cleavage at the proteolytic site separates a non-repetitive hydrophilic amino acid domain from a repetitive unit.

30 29. The silk polypeptide of claim 27, further comprising a first proteolytic site and a second proteolytic site, wherein cleavage at the first proteolytic site and at the second proteolytic site separates the non-repetitive hydrophilic amino acid domain from the repetitive units.

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30. The silk polypeptide of claim 1, further comprising a plurality of non-repetitive hydrophilic amino acid domains wherein the plurality is at least about 2 to about 4 non-repetitive hydrophilic amino acid domains.
- 5 31. The silk polypeptide of claim 1, wherein the non-repetitive hydrophilic amino acid domain is selected from the group consisting of non-repetitive carboxyl terminal regions from MaSpI, MaSpII, ABF-1, ADF-1, ADF-2, ADF-3, ADF-4, and Flag.
- 10 32. The silk polypeptide of claim 1, wherein the non-repetitive hydrophilic amino acid domain is about 20 to about 150 amino acids.
- 15 33. The silk polypeptide of claim 1 further comprising a proteolytic site, wherein cleavage at the proteolytic site results in the separation of the non-repetitive hydrophilic amino acid domain from a repetitive unit.
34. The silk polypeptide of claim 1 further comprising a proteolytic site, wherein cleavage at the proteolytic site results in the separation of the non-repetitive hydrophilic amino acid domain from the repetitive units.
- 20 35. The silk polypeptide of claim 34, wherein the proteolytic site is subject to cleavage by a protease.
36. The silk polypeptide of claim 34, wherein the proteolytic site is subject to cleavage by chemical treatment.
- 25 37. The silk polypeptide of claim 1 further comprising a secretory signal peptide sequence.
38. The silk polypeptide of claim 1 further comprising a c-myc epitope.
- 30 39. The silk polypeptide of claim 1 further comprising a histidine tag.
40. The silk polypeptide of claim 1, wherein the silk polypeptide has a molecular weight between about 16,000 daltons and about 800,000 daltons.
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41. The silk polypeptide of claim 1 wherein the silk polypeptide precipitates and redissolves in an aqueous buffer.
42. A isolated polynucleotide encoding the silk polypeptide as in any of the claims 1-41.
- 5 43. A isolated polynucleotide comprising a nucleotide sequence encoding more than one repetitive unit in a single open reading frame, wherein the repetitive units are independently selected from the group consisting of repetitive units of ADF-1, ADF-2, ADF-3, ADF-4, ABF-1, MaSpI, MaSpII, MiSpI, MiSpII, and Flag.
- 10 44. The polynucleotide of claim 43 wherein the sequences encoding the repetitive units are oriented in their native 5' to 3' direction.
45. A vector comprising the polynucleotide of claim 42.
- 15 46. An expression vector comprising the polynucleotide of claim 42 and a promoter operably linked to coding sequence of the silk polypeptide.
- 20 47. The expression vector of claim 46 wherein the promoter is a tissue-specific promoter selected from the group consisting of uromodulin promoter, uroplakin I, II, and III promoters, rennin promoter, WAP promoter, β -casein promoter, α S1-casein promoter, α S2-casein promoter, κ -casein promoter, β -lactoglobulin, and α -lactalbumin promoter.
- 25 48. A host cell comprising the expression vector of claim 46.
49. The host cell of claim 48, wherein the host cell is a prokaryotic cell.
50. The host cell of claim 48, wherein the host cell is a plant cell.
- 30 51. The host cell of claim 48, wherein the host cell is a yeast cell.
52. The host cell of claim 48, wherein the host cell is a eukaryotic cell.
- 35 53. The host cell of claim 48, wherein the host cell is a mammalian cell.

54. The host cell of claim 48, wherein the host cell is a mammalian epithelial cell.
55. The host cell of claim 54, wherein the host cell is a MAC-T or a BHK cell.
56. A host cell transformed or transfected with the expression vector of claim 47.
57. A host cell that constitutively secretes the silk polypeptide of any of claims 1-41.
58. A host cell that has a polynucleotide integrated into its genome, wherein the polynucleotide encodes the silk polypeptide of any of claims 1-41.
59. The host cell of claim 58, wherein the host cell further comprises a polynucleotide encoding a protease that cleaves at a proteolytic site the silk polypeptide of any of claims 1-41.
60. The host cell of claim 59, wherein the protease is heterologous to the host cell.
61. The host cell of claim 48, wherein the host cell co-expresses a plurality of silk polypeptides.
62. A non-human transgenic mammal that secretes into its urine a silk polypeptide of any claim 1-41.
63. The non-human transgenic mammal of claim 62, wherein the mammal is a ruminant.
64. The non-human transgenic mammals of claim 62, wherein the mammal is a goat.
65. A non-human lactating female transgenic mammal that expresses a silk polypeptide of any claim 1-41 in its milk.
66. The non-human transgenic mammal of claim 65, wherein the mammal is a ruminant.
67. The non-human transgenic mammals of claim 65, wherein the mammal is a goat.

68. A lactating female goat that expresses the silk polypeptide of claim 33, wherein the proteolytic cleavage occurs before the silk polypeptide purified from the milk.

5 69. A method of producing the silk polypeptide of any of claims 1-41 in a host cell comprising:

(A) culturing in cell culture media a host cell containing a nucleic acid encoding the silk polypeptide of any of claims 1-41 under conditions that cause the host cell to express the silk polypeptide; and

10 (B) purifying the silk polypeptide of any of claims 1-41 from the host cell or from the cell culture media.

70. The method of claim 69, wherein the eukaryotic host cell is a mammalian epithelial cell.

15 71. The method of claim 70, wherein the mammalian epithelial cell is a MAC-T cell or a BHK cell.

72. A method of producing silk polypeptides according to claim 69, wherein the non-repetitive hydrophilic amino acid domain is cleaved from a repetitive unit.

20 73. A method of producing silk polypeptides according to claim 69, wherein the non-repetitive hydrophilic amino acid domain is cleaved from the repetitive units.

74. A method of producing the silk polypeptide of any of claims 1-41 comprising:

25 (A) expressing a silk polypeptide of any of claims 1-41 in a transgenic non-human animal; and

(B) recovering the silk polypeptide from a biological fluid produced by the transgenic animal.

30 75. The method of claim 74, wherein the non-human transgenic animal is a female mammal, and the biological fluid is milk.

76. The method of claim 74, wherein the biological fluid is urine.

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77. A method of producing silk polypeptides according to claim 74, wherein the non-repetitive hydrophilic amino acid domain is cleaved from a repetitive unit.

5 78. A method of producing silk polypeptides according to claim 74, wherein the non-repetitive hydrophilic amino acid domain is cleaved from the repetitive units.

79. A method of producing a biofilament comprising a plurality of one or more silk polypeptides, said method comprising:

10 (A) culturing a host cell that expresses a plurality of one or more silk polypeptide of any of claims 1-41 in a transformed or transfected host cell or in a biological fluid of a transgenic ruminant;

15 (B) purifying the plurality of one or more silk polypeptide expressed in step (A);

(C) spinning the plurality of one or more silk polypeptide obtained in step (B) to form a biofilament.

20 80. The method of claim 79, wherein the non-repetitive hydrophilic amino acid domain has been removed from the one or more silk polypeptide before the silk polypeptide is spun in step (C).

25 81. The method of claim 79, wherein the plurality of one or more silk polypeptides consists of about 8 to about 1,000 silk polypeptides.

82. A method of producing a biofilament comprising a plurality of one or more silk polypeptides, said method comprising:

30 (A) expressing a plurality of one or more silk polypeptide of any of claims 1-41 from a transgenic plant or non-human animal;

(B) purifying the plurality of one or more silk polypeptide of any of claims 1-41 from a plant extract or exudate, or from a biological fluid of the non-human animal;

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- (C) spinning a biofilament from the plurality of one or more silk polypeptide of any of claims 1-41 recovered in step (B).

83. A method of producing a biofilament comprising:

- (A) expressing in a host cell or transgenic animal a silk polypeptide comprising a plurality of repetitive units, a non-repetitive hydrophilic amino acid domain, and a proteolytic site operably linked to the non-repetitive hydrophilic amino acid domain such that cleavage at the proteolytic site results in the separation of the non-repetitive hydrophilic amino acid domain from the plurality of repetitive units;
- (B) purifying the silk polypeptide;
- (C) spinning the biofilament from a solution comprising a portion of the silk polypeptide of step (A) remaining after the non-repetitive hydrophilic amino acid domain has been removed by cleavage at the proteolytic site.

84. The method of producing a biofilament of claim 83, wherein said recombinant silk polypeptide has a molecular weight between about 58,000 daltons to about 800,000 daltons.

85. The method of producing a biofilament of claim 83, wherein the toughness of the biofilament is between about 0.6 grams per denier (gpd) and about 1.4 gpd.

86. The method of producing a biofilament of claim 83, wherein the tenacity of the biofilament is between about 1.7 gpd and about 8.0 gpd.

87. A biofilament comprising a plurality of the silk polypeptides of claim 1.

88. A biofilament comprising a plurality of the silk polypeptides produced according to the method of claim 79.

89. A biofilament comprising a plurality of the silk polypeptides produced according to the method of claim 83.